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Novel Avian Reoviruses Capable of Immediate Growth on Mammalian Cells

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Title

Novel Avian Reoviruses Capable of Immediate Growth on Mammalian Cells

Field of the Invention

The present invention is related to novel avian reoviruses that are able to grow in mammalian cells, without adaptation.

Related Applications

This application claims priority from PCT Application No. PCTUS03/31901, titled "Methods of treating and preventing Neurological Symptoms Caused by Avian Reovirus and Novel Associated Characteristics," filed October 9, 2002; provisional application no. 60/417,245, filed on October 9, 2002; provisional application no. 60/418,589, filed on October 15, 2002, provisional application no. 60/424,163, filed on November 6, 2002; and, provisional application no. 60/435,192, filed on December 20, 2002.

Background of the Invention

Reovirus infections are prevalent worldwide in poultry and have been isolated from chickens showing a wide variety of clinical signs including viral arthritis/tenosynovitis, malabsorption syndrome (MAS), pericarditis, myocarditis, and immunosuppression [McNulty, Virus infection of birds, pp181-193 (1993)]

Viral arthritis/tenosynovitis particularly in broiler breeds is the most important disease attributable to reovirus infection. [Nibert et al., Fields Virology 4: p. 1681 (2001)]. It is a persistent viral infection with chronic inflammatory lesions, confined mainly to the hock joints and leg tendons of the chicken. The lesions are localized to synovial structures [Menendez et al., Avian Dis. 19: 112-117 (1975); Ellis et al, Avian Dis. 27: 644-651

(1983); Kibenge et al, Avian Pathol. 14: 87-98 (1985)]; [Nibert et al., Fields Virology 4: p. 1681-1682 (2001)].

Reovirus have also been associated with malabsorption syndrome (MAS) by several groups [Kouwenhoven et al., Avian Pathol. 7: 183-187 (1978); Page et al., Avian Dis. 26:618-624 (1982); Hieronymus et al., Avian Dis. 27: 255-260 (1983) ; Goodwin et al, Avian Dis. 37: 451-458 (1993)]. The syndrome is characterized by weight gain depression with non-uniform growth, defective feathering and diarrhea with undigested food and watery content. The pathologic changes mostly include proventriculitis enteritis, pancreatitis and hepatitis. MAS may be caused either by maldigestion or malabsorption or both. Lesions that are found can cause an impairment of digestion by insufficiency of digestive secretions and or an impairment of absorption because of insufficient absorptive capacity. However, there are a variety of pathogenicities of reovirus isolated from MAS as can been seen in the variations of depressed weight gains. [Nibert et al., Fields Virology 4: p. 1681-1682 (2001); Page et al., Avian Dis. 26:618-624 (1982); McNulty et al., Avian Pathol. 13: 429-439 (1984); Decaesstecker et al., Avian Pathol. 15: 769-782 (1986); Kibenge and Dhillon, Avian Dis. 31: 39-42 (1987); Kouwenhoven et al., Avian Pathol. 17: 879-892 (1988); Montgomery et al., Avian Dis. 41: 80-92 (1997) Songserm et al., Avian Dis. 46: 87-94 (2002)]. To date, there has been no disclosure in the prior art of an avian reovirus causing neurological symptoms.

There are several prior art vaccines for certain strains. Examples of live vaccines include strain S1133 and 2177. Live vaccines in the United States have been developed from various passage levels of one avian reovirus strain, S1133, isolated and characterized by van der Heide from a field case of tenosynovitis. Strain S1133 was grown serially 235

times in the chorioallantoic membrane (CAM) at 37.degree. C. and then 65 times in chicken embryo fibroblast (CEF) at 32.degree. C. An additional 135 passages were carried out at 37 °C in CEF [van der Heide et al., Avian Dis., 27:698-706 (1983)]. Strain 2177 was isolated and characterized by Rosenberger from a field case of avian reovirus [U.S. Pat. No. 5,525,342]. Strain 2177 was isolated from chickens exhibiting reovirus disease by inoculating tissue samples into embryonated eggs and collecting the yolk fluid. This yolk fluid was then inoculated into CEF and passed 14 times until the cytopathology was observed at which time the virus was plaque purified. This yolk fluid was then propagated in embryonated eggs to produce a stock of virus. The stock was found to be non-pathogenic and was titled strain 2177. To date, no chicken reovirus has been able to be grown to suitable titer on Vero cells without adaptation. [Nibert et al., Fields Virology 4: pp. 1681-1682 (2001)]. The most common method is propagation in embryonated chicken eggs. There is disclosure in the prior art of an orthoreovirus from an Australian flying fox, a mammalian reovirus, having some characteristics of an avian orthreovirus (reovirus) that is able to be propagated in mammalian cells. [Nibert et al., Fields Virology 4: p. 1682 (2001)]. As of 2001, there was no disclosure in the prior art of a reovirus that is able to be propagated in a mammalian cell without adaptation. [Nibert et al., Fields Virology 4: p. 1682 (2001)]. Avian reovirus can grow in a variety of primary avian cells from the lung, kidney, liver, macrophage, or testicle of 2 to 6 week old chickens however primary embryo liver cells are preferred. Avian reovirus can be adapted to replicate in chicken embryo fibroblast (CEF) cells. Avian reovirus has been grown on established non-avian cell lines, albeit poorly [S. P. Sahu and N. O. Olson (1975). American Journal of

Veterinary Research, Vol. 36, pp. 847-850; V. Barta, et al. (1984). Avian Diseases, Vol. 28, pp. 216-223].

However, in August of 2001, in Newark, Delaware, at the University of Delaware, a PEMS (Poult enteritis and mortality syndrome) associated astrovirus turkey isolate was disclosed as growing on Vero cells, without adaptation. However, to date, no chicken infecting reovirus has been shown to grow on Vero cells, without prior adaptation. Accordingly, the art field is in search of an chicken reovirus that can grows to suitable titer on Vero cells, without prior adaptation.

Recently, Adriaan van Loon et al [Veterinary Quarterly, 23: 129-133 (2001)] described the isolation and identification of a new class of reoviruses, the so-called enteric reovirus strains (ERS). ERS was isolated from broilers in Poland showing high mortality. Those broilers came from parent flocks that were well vaccinated against reovirus infections. It became clear that ERS was a new serotype of reovirus since the virus could not be neutralized by known reoviurses using the plaque reduction test [van Loon et al., Veterinary Quarterly, 23: 129-133 (2001)]. Furthermore, characterization of ERS with a panel of monoclonal antibodies revealed that these strains showed a distinct panel pattern compared to reovirus strains that have been described in literature [Hieronymus et al., Avian Dis. 27: 246-254 (1983); Johnson, Avian Dis. 16: 1067-1072 (1972); Olson et al., Am J Vet Res. 18: 735-739 (1957); Rosenberger et al, Avian Dis. 33: 535-544 (1989); van der Heide et al., Avian Dis. 18: 289-296 (1974)]. The novel antigenic class of reovirus was further identified by the art-accepted practice of a plaque reduction assay. An explanation of a plaque reduction assay may be found in an article by Nersessian et al. (J. Vet. Res. N50, 1989, pp. 1475-1480). The article confirms the heterogenic immunological character

of avian reovirus and validates the use of plaque reduction assays for determining and characterizing antigenic relationships (including, but not limited to, similarities and differences) between reovirus isolates. Upon screening for Reoviruses in the field, it was observed that ERS type strains were also present in The Netherlands, Belgium, Ireland, United Kingdom, Spain, Germany, Italy, USA, Argentina, United Arabic Emirates, South Africa, The Philippines and Indonesia. These ERS type strains were usually isolated from birds with MAS. Studies of the pathogenicity and dissemination of ERS in specific pathogen free (SPF) chickens demonstrated that ERS was able to induce a high mortality, tenosynovitis (unpublished observations) and MAS [van Loon et al., Veterinary Quarterly, 23: 129-133 (2001)]. Also in commercial broilers with maternally derived antibodies against reovirus, van Loon et al [Veterinary Quarterly, 23: 129-133 (2001)] showed a growth retardation of respectively 35% and 25% in broilers inoculated at day old or at 7 days old.

Summary of the Invention

Without limitation, various embodiments of the present invention generally relate to a class of reoviruses that can be isolated from poultry and grow to suitable titer on Vero cells, without prior adaptation. Various, non-limiting embodiments, of said class comprise, but are not limited to, strain ERS 1037, deposited at the ATCC, Manassas, VA 20108, U.S.A. on October 1, 2002, under accession no. pta-4735; strain ERS 060E, deposited at the ATCC, Manassas, VA 20108, U.S.A. on October 30, 2002, under accession no. pta-4782; and, strain ERS 074, deposited at the ATCC, Manassas, VA 20108, U.S.A. on October 30, 2002, under accession no. PTA-4783.

Further embodiments of the invention comprise a vaccine and/or an immunogenic composition comprising a reovirus of the class of reovirus that can be isolated from poultry and grow to suitable titer on Vero cells, without prior adaptation. Exemplary, non-limiting, strains for use in a vaccine and/or immunogenic composition of the present invention comprise strains ERS 1037, ERS 060E, and ERS 074. Such strains may be used in a live, attenuated and/or inactivated form. Further, such vaccines may further comprise a pharmaceutically acceptable carrier or diluent. Other embodiments further comprise an adjuvant.

Various vaccines and/or immunogenic compositions of the present invention may be administered alone or in combination with other viral vaccines for poultry, such as those for Marek's Disease Virus, Infectious Bursal Disease Virus, Newcastle Disease Virus, Infectious Bronchitis Virus, Avian Encephalomyelitis Virus, Fowl Pox Virus,Chicken Anemia Agent and/or the like.

Administration of immunogenic compositions and/or vaccines comprising embodiments of the present invention may be by any route. Preferred routes of administration include mass application routes, such as drinking water and spray. However, other embodiments of administration may include injection and the like.

Nothing in this summary should be construed as limiting the scope of the invention. For a further understanding of the scope of invention, attention should be had to the following detailed description and claims.

Detailed Description of the Invention

As used herein, the term "relatively non-pathogenic" means and refers to a significant reduction in the pathology of a virus, such that less than or equal to about 20% of poultry immunized with a live strain of the virus would be affected by the virus. As used herein, the term "chicken" means and refers to all chickens, including broilers, reproduction stock, laying stock and the like. The term "poultry," includes, but is not limited to, chickens, turkeys, water fowl, guineas, quail, pigeons, ostriches, and the like. As used herein, the term "naturally" means without human intervention. However, it is contemplated that a "naturally non-pathogenic" strain may be passaged in various embodiments to prepare a virus stock and the like. It is further contemplated that such passaging does not cause a virus to be considered other than "naturally non-pathogenic" if the virus was "non-pathogenic," as defined, before passaging.

As used herein, the term "vaccine strain" means and refers to a viral strain suitable for use in an immunogenic composition or vaccine. A "vaccine strain" can include, but is

not necessarily limited to, a non-pathogenic strain, a killed strain, and/or an attenuated strain.

Accordingly, various embodiments of the present invention generally relate to a class of reoviruses that can be isolated from poultry and grow to suitable titer on Vero cells, without prior adaptation. In an embodiment, methods of the present invention comprise administering an effective amount of an immunogenic composition and/or vaccine comprising an avian reovirus that can be isolated from poultry and grow to suitable titer on Vero cells, without prior adaptation, in a live, attenuated or killed form and a carrier or diluent. Further embodiments comprise an adjuvant.

Avian reoviruses for use with methods of the present invention may be characterized by (i) reactivity in an immuno-fluorescence-technique (IFT) with a polyclonal antiserum raised against an avian reovirus isolate, such as reovirus strain 1133 and/or 2408, (ii) the absence of reactivity in an IFT with Moabs INT 13-06, INT 14-11, and 15-01 INT (samples of which are deposited at the ECACC under accession nos. 99011472, 99011473, and 9901474, respectively), and/or, (iii) that can be isolated from poultry and grow to suitable titer on Vero cells, without prior adaptation. In an embodiment, all three characterizations are present.

Exemplary, non-limiting, strains representative of reoviruses suitable for use in methods of the present invention include, but are not limited to, strain ERS 1037, deposited at the ATCC, Manassas, VA 20108, U.S.A. on October 1, 2002, under accession no. pta-4735; strain ERS 060E, deposited at the ATCC, Manassas, VA 20108, U.S.A. on October

30, 2002, under accession no. pta-4782; and, strain ERS 074, deposited at the ATCC, Manassas, VA 20108, U.S.A. on October 30, 2002, under accession no. PTA-4783.

Strains used in immunogenic compositions or vaccines may be isolated from poultry as is common in the art. For chickens, a strain of reovirus is isolated from chickens with reovirus associated disease. Processes for virus isolation are common in the art. In an isolation, the virus was isolated from infected tendons collected from broilers showing signs of reovirus associated disease. However, methods of the present invention are not limited by from what part of avian the virus is isolated.

In fact, surprisingly, it has been found that certain embodiments of an avian reovirus of the present invention may be isolated from the brain and spinal chord. Avian reovirus has not previously been isolated from the brain, spinal chord, and/or other structures associated with the neurological system. However, said class is not limited to an avian reovirus that can be isolated from the neurological system.

In various embodiments of the present invention relate to an antigenic class of reoviruses that can grow to suitable titer on Vero cells, without prior adaptation, wherein suitable titer is at least about 3.0 $TCID_{50}$/ml. In an alternate embodiment, the titer is at least about 4.0 $TCID_{50}$/ml. In a separate embodiment, the titer is at least about 5.1 $TCID_{50}$/ml. In another embodiment, the titer is at least about 5.3 $TCID_{50}$/ml.

Immunogenic compositions and/or vaccines used with methods of the present invention may be administered alone or in combination with other viral vaccines for

poultry, such as those for Marek's Disease Virus, Infectious Bursal Disease Virus, Newcastle Disease Virus, Infectious Bronchitis Virus, Avian Encephalomyelitis Virus, Fowl Pox Virus,Chicken Anemia Agent and/or the like. Other embodiments may combine bacterial antigens and the like. Further preferred immunogenic compositions or vaccines used with methods of the present invention are vaccines which may be administered at a young age (e.g., one day) and/or *in ovo*.

Administration of immunogenic compositions or vaccines in methods of the present invention may be by any route commonly used for avian reovirus administration Preferred routes of administration include mass application routes, such as drinking water (orally) and spray. However, other embodiments of administration may include injection and the like.

It is common in administration by the drinking water route to deprive the animals of water for about 2 to 4 hours before placing the immunogenic composition or vaccine containing water before them. It is important that there is enough drinker space for all birds to drink evenly to allow for even vaccine dispersion. The vaccine is applied in fresh drinking water at an effective amount, a concentration calculated to give each bird a sufficient dose for an immunogenic composition or vaccine.

In order to prevent a reduction of the viable vaccine virus by the presence of small amounts of chlorine, iron, zinc or copper ions in the drinking water and/or to prevent inactivation of the live vaccine virus because of increased concentration of dissolved salts as a result of desiccation of the drinking water, small amounts of a protein protectant, such as skimmed milk, skimmed milk powder or gelatin can be added to aqueous phase.

In various methods utilizing a spray, common methods of application include, but are not limited to, a coarse spray application and an aerosol administration. In the coarse spray method particles usually have an initial droplet size ranging from 10 to 100 microns and in the aerosol administration method droplets usually range from <1 to 50 microns. However, any droplet size may be used in varying methods associated with the invention.

It is common in the industry to utilize conventional spray-apparatus and aerosol generators for the generation of the small particles, such as the commercially available spray generators for knapsack spray, hatchery spray and atomist spray. Details concerning conventional spray/aerosol- and drinking water vaccination can be found in the "Compendium, administration of poultry vaccines" issued by the Gezondheidsdienst voor Pluimvee, Doorn, The Netherlands, van Eck et al., VI-VII, 1988.

Further methods of administration of vaccines of the present invention may utilize administration through eye drop and/or beak dipping and/or any other method common in the art.

Various embodiments of the present invention comprise an inactivated avian reovirus. Inactivated forms provide a benefit of elevated levels of protective antibodies for a long duration. This property makes an inactivated vaccine particularly well suited for breeder vaccination.

As is known in the art, an aim in the inactivation of viruses harvested after propagation is to eliminate reproduction of viruses. In general, this can be achieved by chemical or physical means. Chemical inactivation can be effected by treating the viruses with, for example, enzymes, formaldehyde, β-propiolactone, ethylene-imine, or a derivative thereof. If necessary, the inactivating material is neutralized afterwards. For example,

material inactivated with formaldehyde can be neutralized with thiosulphate. Physical inactivation can preferably be carried out by subjecting the viruses to energy-rich radiation, such as UV light or γ-rays. If desired, after treatment the pH can be adjusted to a value of about 7.

Inactivated vaccines may be administered parent rally, e.g. intramuscularly or subcutaneously.

Further embodiments of the present invention comprise a method of propagating an avian reovirus that can be isolated from poultry and grow to suitable titer on Vero cells, without prior adaptation, comprising the steps of:

a. inoculating a Vero cell with the avian reovirus that can be isolated from poultry and grow to suitable titer on Vero cells, without prior adaptation;
b. allowing the avian reovirus to multiply; and,
c. harvesting the avian reovirus.

Exemplary, non-limiting strains for use in methods of then present invention include, but are not limited to ERS 1037, ERS 060E, and ERS 074.

Unexpectedly, had a titer of at least about 5.1/ml. Yet further embodiments had a titer of at least about 5.3/ml. However, the titer will vary depending on the strain used and the growth conditions. A titer of at least about 3.0 $TCID_{50}$/ml is contemplated in embodiments of the present invention. However, lower titers may be achievable depending upon the growth conditions and needs.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and the appended Claims are intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth whether now existing or after arising. Further, while embodiments of the invention have been described with specific dimensional characteristics and/or measurements, it will be understood that the embodiments are capable of different dimensional characteristics and/or measurements without departing from the principles of the invention and the appended Claims are intended to cover such differences. Furthermore, all patents, patent applications, articles, and other publications mentioned herein are herby incorporated by reference.

For a further understanding of various embodiments of the present invention, reference should be had to the following examples:

Examples:

Samples from flocks of chickens in the United States of America were taken and strains were collected from the flock. Certain of these strains were identified as ERS 060E, ERS 074, and ERS 1037. The flocks from which these chickens were taken were experiencing classic symptoms of ERS, including, but not limited to enteritis. As well, strains from these flocks were experiencing neurological symptoms. Serum from chickens

infected with these strains was sent to Boxmeer, The Netherlands, for a panel pattern. All three sera illustrated the absence of reactivity in an IFT with Moabs INT 13-06, INT 14-11, and 15-01 INT.

Based upon the results comprising the study, it was determined that strains ERS 1037, ERS 060E, and ERS 074 belonged to the novel antigenic class of reoviruses identified as ERS in U.S. application 09/493,484.

Further studies of strains ERS 060E and ERS 074 revealed that the novel antigenic class of reoviruses causes neurological symptoms in poultry infected with the isolated reovirus. Such neurological symptoms including, but not being limited to twisted neck, tremors and/or the like. The observations were made in both SPF chickens and commercial broilers, with maternally derived antibodies against reovirus. Reference to the following experiments illustrates the specific results.

Example 1a:

Ongoing studies have revealed that strains of avian reovirus causing neurological symptoms can be isolated from the brain and/or spinal chord.

In one example, ERS 074 was reisolated from SPF Chickens' brains and spinal chords using the following procedure:

1. Chickens showing CNS signs were necropsied and the head and necks were collected and frozen.
2. Heads and necks were thawed.

3. Brain and upper portion of the spinal cord were asceptically swabbed.

4. Swabs were placed in TPB and vortexed.

5. Samples were frozen/thawed three times.

6. Samples ere centrifuged for 10 minutes at approx. 3000 rpm.

7. Supernatant was removed and filtered through 0.45μm and 0.2μm syringe filters.

8. 0.1 ml of filtered material was inoculated into 10 day eggs using the drop CAM route.

9. Eggs were candled daily for death.

10. Six days post inoculation all eggs were opened.

11. Liver and chorioallantoic membranes were harvested from infected embryos.

12. Liver and chorioallantoic membranes were homogenized using a Waring blender.

13. Homogenates were frozen and thawed three times.

14. An AGP(Agar Gel Precipitin Test) was used to detect avian reovirus antigen.

The AGP test was positive for avian reovirus antigen.

Example 1b:

Ongoing studies have further revealed that strains ERS 1037, ERS 060E, and ERS 074 (the ERS US isolates) can be isolated from poultry and grow to suitable titer on Vero cells, without prior adaptation. This trait is unexpected because prior art avian reoviruses have required special adaptation for growth on Vero cells.

Growth on Vero cells, without prior adaptation was observed with these strains following this procedure:

1. 60mm tissue culture (TC) microtiter dish was seeded with Vero cells (1.3 x 10^6/dish).
2. TC dish was inoculated with 0.2 ml of ground tendon homogenate from the US isolates in a ten fold serial dilution.

3. CPE was observed 4-5 days PI.

The titer results are presented in the following table:

Sample	Dilution:					TCID 50/ml
	-1	-2	-3	-4	-5	EID 50/ml
ERS 074	10	10	10	10	1	5.3/ml
ERS060E	10	10	10	7	2	5.1/ml

Surprisingly, these US isolates are able to be isolated from poultry and grow to suitable titer on Vero cells, without prior adaptation. Prior art reoviruses have either required adaptation or growth in embryonated chicken eggs prior to growth on Vero cells.

Example 2 (Panel Pattern):

Polyclonal antiserum was prepared by infecting rabbits (1-1.5 kg) with purified avian reovirus strain 1133. Booster injections took place 28 and 84 days after the first injection. Blood was collected and serum isolated 14 days after the last injection.

Different reovirus were characterized with different Moabs. In an embodiment, primary CEL cells were grown in 96-well polystyrene microtitre plates. Uninfected cells served as controls. After 2-4 days of incubation at 37°C with 5% CO_2, infected monolayers were fixed with cold 96% ethanol. The alcohol was discarded and the plates were washed with washing buffer and 100 µl of different hybridoma cell culture supernatant diluted 1:50 or 1:200 in PBS or 100 µl of rabbit polyclonal serum (rabbit 68A) diluted 1:50, was added to each well. The plates were incubated for 60-90 minutes at 37°C, washed twice with washing buffer and reacted with 1:100 diluted fluorescent isothiocyanate-labelled rabbit anti-mouse or 1:100 diluted isothiocyanate-labelled goat anti-rabbit serum. The plates

were then washed and fixed with a glycerol/PBS solution (1:1). The presence of fluorescence was observed with a fluorescence microscope.

The antiserum panel used in this experiment consisted of the following polyclonal antiserum and Moabs raised against the prototype avian reovirus strain 1133:

Rabbit 68A	rabbit polyclonal antiserum
Moab 154	Vakharia et al. 1996 (supra)
Moab 14-67	Intervet International B.V.
Moab INT 13-06	ECACC accession no. 99011472
Moab INT 14-11	ECACC accession no. 99011473
Moab 15-01 INT	ECACC accession no. 99011474

The panel pattern was as follows:

Strain	Rabbit 68A	154	14-67	14-11	13-06	15-01
S-1133	±	±	±	±	±	±
2408	±	±	±	±	±	±
1733	±	±	±	±	±	-
2177	±	±	±	-	-	±
ERS(isolate 1)	±	±	±	-	-	-
ERS 1037	±	±	±	-	-	-
ERS 060E	±	±	±	-	-	-
ERS 074	±	±	±	-	-	-

As can be seen from the panel patterns, strains ERS 1037, ERS 060E, and ERS 074 have a comparable pattern to strain ERS (isolate 1) while being differentiated from strains S-1133, 2408, 1733, and 2177.

Example 3:

The following experiments demonstrate studies conducted with various identified strains.

Experiment 3a

Experimental design

Twenty SPF chickens were inoculated orally at day old with 0.5ml plaque purified strain ERS (isolate 1) ($6.09\log_{10}TCID_{50}$/bird). The chickens were observed daily for clinical sign during 14 days. At 14 days of age all chickens were slaughtered.

Results

35% of the chickens died during the first 6 days (Table 1). At day 10, one chicken developed neurological symptoms and at day 13 a second chicken showed neurological symptoms. Further, chicks infected with ERS demonstrated a 10.1 gram/day weight gain, while the control group experienced a 17.0 gram/day weight gain.

Table 1. Percentage mortality

Days after inoculation	Mortality (number birds)	% Mortality
3	1	5
4	2	15
5	3	30
6	1	35

Table 1. Percentage neurological symptoms

Days after inoculation	Neurological Symptoms (number birds/remaining birds)*	% NS
10	1/13	7.7
13	1/13	15

* A chicken was determined to be showing neurological symptoms when the chicken was observed with twisted neck and/or tremors.

Experiment 3b

10 SPF chickens were inoculated subcutaneously with 0.2 ml at one day of age with strain ERS 060E (10^3 $TCID_{50}$/bird). The chickens were observed for clinical sign at 15 days.

Table 1. Percentage mortality

Days after inoculation	Mortality (no. of birds)	% Mortality
20	2	20

Table 1. Percentage neurological symptoms

Days after inoculation	Neurological Symptoms (number birds)*	% NS
20	4	40

*A chicken was determined to be showing neurological symptoms when the chicken was observed with twisted neck and/or tremors.

Results:

20% of the birds died during the first 15 days. 40% of the birds suffered from neurological symptoms. Further, chicks infected with ERS 060E demonstrated an average 93.82 gram/day weight gain.

Experiment 3c

10 SPF chickens were inoculated subcutaneously with 0.2 ml at one day of age with strain ERS 074 (10^3 $TCID_{50}$/bird). The chickens were observed for clinical sign at 20 days.

Table 1. Percentage mortality

Days after inoculation	Mortality (no. of birds)	% Mortality
20	3	30

Table 1. Percentage neurological symptoms

Days after inoculation	Neurological Symptoms (number birds)*	% NS
20	0	0

* A chicken was determined to be showing neurological symptoms when the chicken was observed with twisted neck and/or tremors.

Results:

30% of the birds died during the first 20 days. 0.0% of the birds suffered from neurological symptoms. Further, chicks infected with ERS 074 demonstrated a 126.38 gram/day weight gain.

Experiment 3d

10 SPF chickens were inoculated via the foot pad route with 0.2 ml at one day of age with strain ERS 060E (10^3 $TCID_{50}$/bird). The chickens were observed for clinical sign at 20 days.

Table 1. Percentage mortality

Days after inoculation	Mortality (no. of birds)	% Mortality
20	2	20

Table 1. Percentage neurological symptoms

Days after inoculation	Neurological Symptoms (number birds)	% NS
20	1	10

* A chicken was determined to be showing neurological symptoms when the chicken was observed with twisted neck and/or tremors.

Results:

20% of the birds died during the first 20 days. 10% of the birds suffered from neurological symptoms. Further, chicks infected with 060E demonstrated a 87.81 gram/day weight gain.

Experiment 3e

10 SPF chickens were inoculated via the foot pad route with 0.2 ml at one day of age with strain ERS 074 (10^3 $TCID_{50}$/bird). The chickens were observed for clinical sign at 20 days.

Table 1. Percentage mortality

Days after inoculation	Mortality (no. of birds)	% Mortality
20	10	100

Table 1. Percentage neurological symptoms

Days after inoculation	Neurological Symptoms (number birds)*	% NS
20	5	50

* A chicken was determined to be showing neurological symptoms when the chicken was observed with twisted neck and/or tremors.

Results:

100% of the birds died during the first 20 days. 50% of the birds suffered from neurological symptoms. Further, chicks infected with ERS 074 demonstrated a 94.37 gram/day weight gain.

Experiment 3f

20 broiler chickens were inoculated subcutaneously with 0.2 ml at one day of age with strain ERS 060E (10^3 $TCID_{50}$/bird). The chickens were observed for clinical sign at 14 days.

Table 1. Percentage mortality

Days after inoculation	Mortality (no. of birds)	% Mortality
14	3	15

Table 1. Percentage neurological symptoms

Days after inoculation	Neurological Symptoms (number birds)*	% NS
14	2	10

* A chicken was determined to be showing neurological symptoms when the chicken was observed with twisted neck and/or tremors.

Results:

15% of the birds died during the first 14 days. 10% of the birds suffered from neurological symptoms. Further, chicks infected with ERS 060E demonstrated an average weight of 543 grams at 21 days post-inoculation. Negative control birds had an average weight of 751 grams at 21 days post-inoculation.

Experiment 3g

20 broiler chickens were inoculated subcutaneously with 0.2 ml at one day of age with strain ERS 074 (10^3 $TCID_{50}$/bird). The chickens were observed for clinical sign at 14 days.

Table 1. Percentage mortality

Days after inoculation	Mortality (no. of birds)	% Mortality
14	5	25

Table 1. Percentage neurological symptoms

Days after inoculation	Neurological Symptoms (number birds)*	% NS
14	5	25

* A chicken was determined to be showing neurological symptoms when the chicken was observed with twisted neck and/or tremors.

Results:

25% of the birds died during the first 14 days. 25% of the birds suffered from neurological symptoms. Further, chicks infected with ERS 074 demonstrated an average weight of 488 grams at 21 days post-inoculation. Negative control birds had an average weight of 751 grams at 21 days post-inoculation.

Experiment 3h

20 broiler chickens were inoculated via the foot pad route with 0.2 ml at one day of age with strain ERS 060E (10^2 $TCID_{50}$/bird). The chickens were observed for clinical sign at 14 days.

Table 1. Percentage mortality

Days after inoculation	Mortality (no. of birds)	% Mortality
14	5	25

Table 1. Percentage neurological symptoms

Days after inoculation	Neurological Symptoms (number birds)*	% NS
14	1	5

* A chicken was determined to be showing neurological symptoms when the chicken was observed with twisted neck and/or tremors.

Results:

25% of the birds died during the first 14 days. 5% of the birds suffered from neurological symptoms. Further, chicks infected with ERS 060E demonstrated an average weight of 442 grams at 21 days post-inoculation. Negative control birds had an average weight of 751 grams at 21 days post-inoculation.

Experiment 3i

20 broiler chickens were inoculated via the foot pad route with 0.2 ml at one day of age with strain ERS 074 (10^2 $TCID_{50}$/bird). The chickens were observed for clinical sign at 14 days.

Table 1. Percentage mortality

Days after inoculation	Mortality (no. of birds)	% Mortality
14	10	50

Table 1. Percentage neurological symptoms

Days after inoculation	Neurological Symptoms (number birds)*	% NS
14	1	5

* A chicken was determined to be showing neurological symptoms when the chicken was observed with twisted neck and/or tremors.

Results:

50% of the birds died during the first 14 days. 5% of the birds suffered from neurological symptoms. Further, chicks infected with ERS 074 demonstrated an average weight of 473 grams at 21 days post-inoculation. Negative control birds had an average weight of 751 grams at 21 days post-inoculation.

Experiment 3j: Non-pathogenic Strains

ERS strain 1037 was passed in embryonated eggs for approximately 10 passages to prepare a stock of virus that was used to inoculate 1-day old broiler chickens via the intratracheal route. A study of ERS strain 1037 was conducted against a highly pathogenic reovirus strain 1733.

Reo Strain 1037 versus Reo Strain 1733

Progeny Group	Chall. Strain	# chicks	14 day challenge results			
			# positive (weight)[1]	# positive (mortality)[2]	Total Positive	% Affected
SPF Controls	1733	50	15	35	50	100
	1037	50	10	0	10	20

[1] Determined by t-test analysis
[2] Positive birds showed evidence of Reovirus infection at necropsy

Strain 1037 was shown to be relatively non-pathogenic because only about 20% of the chickens were affected, exhibited any signs of malabsorption syndrome or growth depression, and none of the chickens died during the test period.. This is in contrast to 1733, also a malabsorption virus, that caused 100% of the chickens to be affected, to have weight loss and/or death. Likewise, as can be seen, strain 1733 killed 35% percent of the chicks whereas strain 1037 killed none. Therefore, strain 1037 is non-pathogenic.

While the invention has been described in connection with specific embodiments, it will be understood that it is capable of further modifications and the appended Claims are intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth whether now existing or after arising. Further, while embodiments of the invention have been described with specific dimensional characteristics and/or measurements, it will be understood that the embodiments are capable of different dimensional characteristics and/or measurements

without departing from the principles of the invention and the appended Claims are intended to cover such differences. Furthermore, all patents, patent applications, articles, and other publications mentioned herein are herby incorporated by reference, including those patents, patent applications, articles, and other publications referenced in the patents, patent applications, articles, and other publications mentioned herein.

For a further understanding of the scope of the present invention, consideration should be had to the appended Claims.